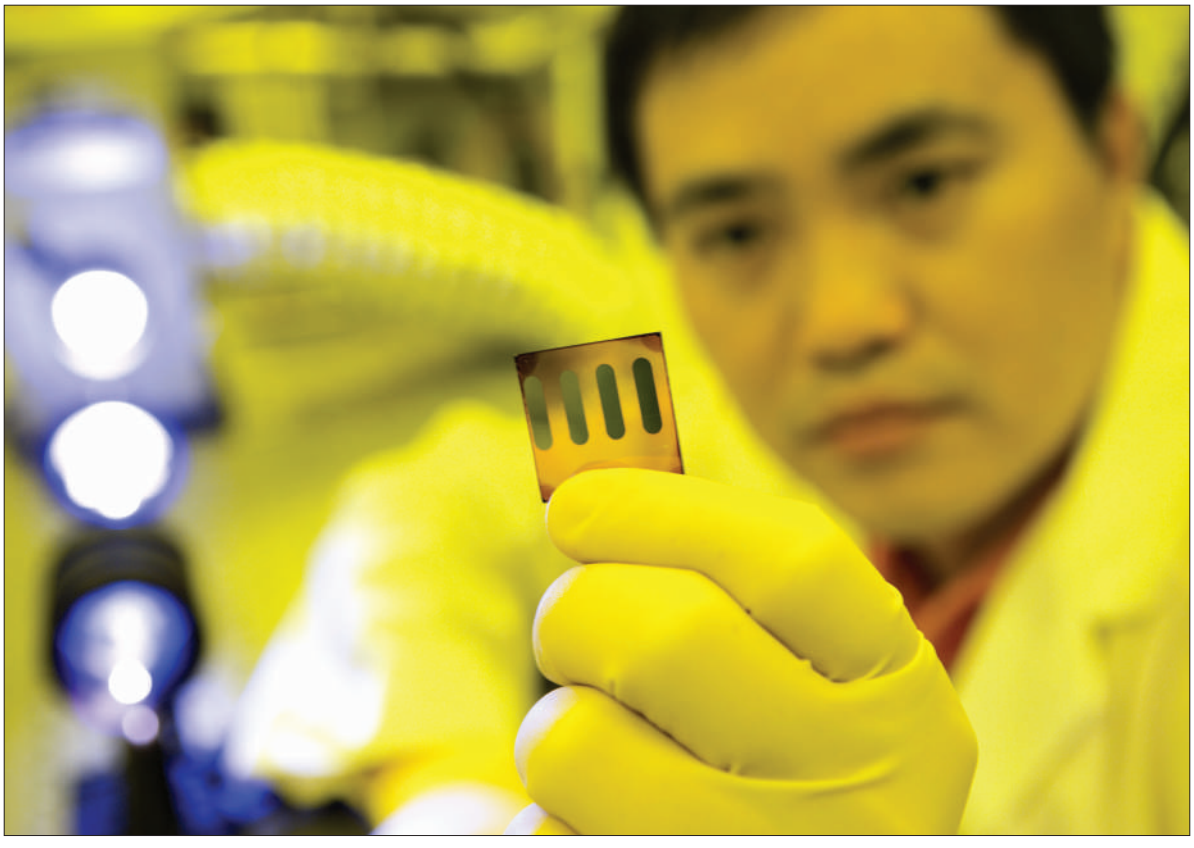

Singapore Journal of

Scientific Research

ISSN: 2010-006x

science alert

http://scialert.net/sjsr

OPEN ACCESS Singapore Journal of Scientific Research

ISSN 2010-006x
DOI: 10.3923/sjsres.2020.105.122

Research Article
Genetic Characterization for Three Groups of Seed Heterospermy for Some Wild Plants

[1]Magda Ibrahim Soliman, [2]Zakria Bakka, [3]Amira Abd Allah Ibrahim and [1]Nerveen Mohamed Essa

[1]Department of Botany, Faculty of Science, Mansoura University, Egypt
[2]Department of Botany, Faculty of Science, Damietta University, Egypt
[3]Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications, Universities and Research Center District, New Borg El-Arab, Alexandria, Egypt

Abstract

Background and Objective: Genetic characterization for seed heterospermy (seed shape, seed size and seed color) has been studied based on cytological (chromosome number), molecular markers (RAPD, ISSR and SCoT) and morphological study. The aim of this study was to clarify the genetic diversity characterization in 6 Egyptian taxa characterized by heterospermy i.e., seed polymorphism either seed shape, seed size and seed color polymorphism. **Materials and Methods:** Seeds and young leaves of three groups: seed shape polymorphism (*Emex spinosa* and *Limbarda crithmoides*), seed size polymorphism (*Cakile martima* and *Raphanus raphanistrum*) and seed color polymorphism (*Chenopodium murale* and *Urospermum picroides*) were collected from their natural habitats in Egypt. Seeds were used for metaphase chromosome number after fixation and squashing steps. Molecular markers RAPD, ISSR and SCoT were used by five primers for each marker. **Results:** In this study chromosome number for *Emexspinosa* 2n = 20, *Limbarda crithmoides* 2n = 18, *Cakilemartima* 2n = 18, *Raphanus raphanistrum* 2n = 18, *Chenopodiummurale* 2n = 18 and 2n = 10 for *Urospermum picroides* were found. RAPD, ISSR and SCoT molecular markers generated a total 492 total bands with 130 polymorphic bands and 362 common bands. RAPD marker was efficient to differentiate among studied taxa giving high polymorphism 33.96%. Scanning Electron Microscope (SEM) had the ability to compare between 2 seeds for the same plant giving different appearance in seed coat. **Conclusion:** Seed heterospermy not differ in chromosome number but differed in chromosome structure. In addition, it differed genetically that appeared in using molecular markers and scanning electron microscope. This attributes have the ability to conserve genetic diversity among species and can be used in the conservation of these species in gene banks.

Key words: Seed polymorphism, chromosome number, RAPD, ISSR, SCoT, SEM

Citation: Magda Ibrahim Soliman, Zakria Bakka, Amira Abd Allah Ibrahim and Nerveen Mohamed Essa, 2020. Genetic characterization for three groups of seed heterospermy for some wild plants. Singapore J. Sci. Res., 10: 105-122.

Corresponding Author: Magda Ibrahim Soliman, Department of Botany, Faculty of Science, Mansoura University, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Seed heteromorphism, seed polymorphism or seed heterospermy is the evolutionary strategy where a plant species produce 2 or more distinct types of seed in single plant, which may differ in several categories like morphology, ripening, dormancy, seed size, dispersal, germination time and characteristics and ability to persist in seed bank and seedling growth[1-3]. Seed heteromorphism is commonly observed in some families such as Asteraceae, Chenopodiaceae, Poaceae and Brassicaceae and has been mostly observed in those species that distributed in semi-arid, saline and other unfavorable conditions[2,4]. Recently, it was founded in 292 species in 129 genera and 26 families[3]. Heterospermy can increase variability in seed dormancy[5], dispersal[6] and competitive ability[7].

It is commonly recorded in a number of plant genera such as *Arthrocnemum*, *Chenopodium*, *Cakile*, *Salicornia*, *Salsola*, *Spergularia*, *Suaeda*, *Trianthema* and *Atriplex*, in which many are halophytes[8] allowing them to adapt to varying salt-marsh or dry salt desert environments. It also enhances chances for seedling establishment and survival in a saline environment[2].

Seed heteromorphism or seed polymorphism is known as the production of seeds that differ in shape, size, color and/or external structure as well as in dispersal, dormancy behavior, germination characteristics, ability to persist in seed bank and seedling growth and often heteromorphic seeds are from different positions on the same plant[9].

Seed size polymorphism is known as the size variations in seeds produced by a species. A sizeable body of knowledge exists on this phenomenon[10]. In many species, seed size variation has important connection to the overall biological fitness of parental species by directly affecting the process of germination, seedling recruitment and competitive ability[11].

Cakile maritima Scop. (Brassicaceae), "sea rocket", is a succulent annual or biennial species narrowly restricted in habitat to the coastal strandline but widely distributed throughout the world[12]. Somatic dimorphisms in *C. martima* consist of two morphological distinct types of fruit segments, upper and lower. The number of seeds in *C. maritime* differs between upper and lower fruit segments[13]. The large number of aborted and multiple seeds in lower (proximal) segments in relation to upper (distal) segments were reported in several works[14].

According to Stanton[15], seed mass in wild radish (*Raphanus raphanistrum*) varies up to 6 fold within single fruits. Seeds from single fruits were used to minimize genetic differences among individuals. Large seeds (>6 mg) were more likely to emerge as seedlings than were small seeds (<4 mg). Seed size had no effect on emergence time and no effect on upon final plant size. Seedlings from large seeds grew more rapidly and produced more flowers than did those from related smaller seeds.

Chenopodium album (Chenopodiaceae) is a weed species with seed heteromorphism, which is widely distributed all over the world including the semi-arid areas[16]. It produces two distinct lots of seeds (morphs), some with a brown seed coat are non-dormant and germinate rapidly after the initial harvest, the majority with a black seed coat that are dormant[17]. The different morphs seeds of *C. album* also differed in phenotypic characters, germination behavior and salinity tolerance. Variation of seed heteromorphism was also observed between populations and such variation might partially be attributed to the salinity in environments. Salinity stress is suggested as one of the environmental factors that may induce the variation in seed heteromorphism of *C. album* in semi-arid and light-saline areas[16].

Cytogenetics has a great role in studies of chromosome number, structure, behavior and evolution in numerous plant species[18]. Cytological techniques are excellent tool to determine the chromosome constitution of organism and facilities recognition of the individual chromosome. Cytological characters including chromosome number and karyotype analysis have been considered as a reliable guide in studies of taxonomic and evolutionary relationships[19].

The molecular markers are not influenced by environmental condition and show higher levels of polymorphism[20]. SCOT polymorphism gains popularity for its superiority over other dominant DNA marker system[21]. In *Raphanus sativa* L., many molecular markers are RAPD[22] and AFLP[23].

Scanning Electron Microscopy (SEM) is a versatile method used widely in identification of different species of genera through examination of different plant parts. Use of SEM in the study of seed coat has been critically reviewed by Brisson and Peterson[24]. Seed morphology provides a number of characters useful for species identification, phylogenetic inference and character state evolution[25]. Polymorphism thus provides a window on the maintenance of genetic diversity in nature. The aim of this study was to enlighten the genetic diversity characterization in six Egyptian taxa characterized by heterospermy i.e., seed polymorphism either seed shape, seed size and seed color polymorphism.

MATERIALS AND METHODS

Plant collection: Three groups of seed polymorphism were chosen: seed shape, seed size and seed color, for each group 2 taxa were collected from their natural habitats in north

Table 1: Location, habitat, soil type and GPS of 3 groups of seed polymorphism in Egypt

Groups	Seed polymorphism	Taxa	Family	Collection area	Governorate	Habitat	GPS		
							Soil type	Longitude (E)	Latitude (N)
A	Seed shape	*Emex spinosa* (L.) campd.	Polygonaceae	Burg EL-Arab	Alexandria	Orchards	Calcareous sandy soil	29.574749°	30.879377°
		Limbarda crithmoides (L.) Dumort.	Asteraceae	New Damietta	Damietta	Dry salt marches	Siliceous sandy soil	31.8144°	31.4175°
B	Seed size	*Cakile maritima* Scop.	Cruciferae (Brassicaceae)	Gamsa-Qalabshu	El-Dakahlia	Coastal road side	Siliceous sandy soil	29.33034°	26.2512°
		Raphanus raphanistrum (L.)	Cruciferae (Brassicaceae)	Burg EL-Arab	Alexandria	Barley fields	Calcareous sandy soil	29.574749°	30.879377°
C	Seed color	*Chenopodium murale* (L.)	Chenopodiaceae	Mansoura University	El-Dakahlia	Neglected lands	Clayey soil	31.3589°	31.0410°
		Urospermum picroides (L.) F.W.	Asteraceae	Mansoura University	El-Dakahlia	Neglected lands	Clayey soil	31.3589°	31.0410°

Mediterranean coast and Delta in Egypt in duration from May-June, 2018. The selected taxa and their locations with their GPS were illustrated in Table 1.

Cytological studies: The seeds from each group was germinated and the root tips were fixed in solution (3 absolute alcohol: 1 glacial acetic acid) (v/v) and kept at 4°C for at least 1 week. Then root tips hydrolysis was performed using 1 N HCL, then staining techniques was used by 2% aceto-orcein[26]. Well spread metaphase plates were selected for recording observation. The microscope used a full automatic Olympus microscope. Slides were scanned using a 100X professional C 400 and digital microscope camera.

Molecular studies

DNA extraction: DNA from young leaves of the studied taxa was extracted using CTAB buffer according to Dellaporta *et al.*[27].

PCR reaction: Three molecular markers (RAPD, ISSR and SCoT) were used to study the genetic diversity among studied taxa. Five primers for each molecular marker were used.

The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 95°C for 3 min followed by 40 cycles of 30 sec at 94°C, 1 min at annealing temperature for each primer and 2 min at 72°C. The reaction was finally stored at 72°C for 10 min. Names of primers for each marker and their sequences, in addition to annealing temperatures were recorded in Table 2.

Scanning Electron Microscope (SEM): For the scanning electron microscope (SEM) study, the seeds were fixed in glutaraldehyde and dehydrated through an alcohol-acetone series. The specimens were then dried using a critical point drying (Autosamdri-815, USA). The dried specimens were mounted on SEM stubs using electrical silver paint and coated with gold-palladium membranes in a coating unit in an atmosphere of argon for 2×2 min, at 45 mA. The specimens were then examined and photographed using a Jeol JSM-6510 L.V SEM. The microscope was operated at 30 KV at EM Unit, Mansoura University, Egypt.

RESULTS

Cytological study: Chromosome number of all studied taxa were recorded in Table 3, for Group A "seed shape polymorphism", 2n = 20 for *Emex spinosa* for aerial seed (Plate 1a, b), for subterranean seed (Plate 1c, d), where

Table 2: Primers name, sequence and annealing temperature of RAPD, ISSR and SCoT molecular markers

Molecular markers	Primers	Sequence (5'-3')	Annealing temperature (°C)
RAPD	OP-A2	GTG ATC GCA G	37
	OP-A07	GAA AGG GGT G	
	OP- B7	GAA AGG GGT G	
	OP-B11	GTA GAC CCG T	
	OP-C9	CTC ACC GTC C	
ISSR	14A	CTC TCT CTC TCT CTC TTG	57
	49A	CAC ACA CAC ACA AG	
	HB-9	CAC CACCAC GC	
	HB-12	CAC CACCAC GC	
	HB-15	GTG GTGGTGGC	
SCoT	SCoT 1	ACG ACA TGG CGA CCA CGC	57
	SCoT 3	ACG ACA TGG CGA CCC ACA	
	SCoT 4	ACC ATG GCT ACC ACC GCA	
	SCoT 8	CAA TGG CTA CCA CTA CAG	
	SCoT 9	ACA ATG GCT ACC ACT ACC	

Table 3: Chromosome number of the studied taxa of seed polymorph

				Numbers	
Groups	Seed polymorphism	Taxa	Seed type	Current chromosome	Previous chromosome
A	Seed shape	*Emexspinosa* (L.) Campd.	Aerial seed	2n = 20	2n = 20 Malallah *et al.*[28]
			Subterranean seed		2n = 18 Abd El-Twab *et al.*[29]
		Limbarda crithmoides (L.)Dumort.	Monomorphic pappus	2n = 18	2n = 18 Abd El-Twab *et al.*[29]
			Dimorphic pappus		
B	Seed size	*Cakile maritima* scop.	Large seed	2n = 18	2n = 18 Soliman[30]
			Small seed		
		Raphanus raphanistrum (L.)	Large seed	2n = 18	2n = 18 Benabdelmouna *et al.*[31]
			Small seed		
C	Seed color	*Chenopodium murale* (L.)	Black	2n = 18	2n = 18 Malallah *et al.*[28]
			Brown		
		Urospermum picroides (L.) F.W.	Black	2n = 10	2n = 10 Abd-El-Wahid *et al.*[32]
			Brown		
			White		

2n = 18 for *Limbarda crithmoides* for seed with monomorphic pappus (Plate 1e) and for seed with dimorphic pappus (Plate 1f). For Group B "seed size polymorphism", 2n = 18 for *Cakile martima*. For large seed (Plate 2a, b), for small seed (Plate 2c, d). Chromosome number 2n = 18 of *Raphanus raphanistrum* for large seed for small seed (Plate 2e, f). For Group C "seed color polymorphism", chromosome number for *Chenopodium murale* 2n = 18 for black seed and for brown seed (Plate 3a, b), where 2n = 10 for *Urospermum picroides* for black seed, brown seed and for white seed (Plate 3c-h).

Molecular studies:

- **Group A "seed shape polymorphism":** For RAPD molecular marker, 5 primers were used to differentiate between seed shape polymorphism. For *Emex spinosa*, there was a total 23 bands with 11 polymorphic bands, 12 common bands and 11 unique bands. Regarding for ISSR marker there are 28 total bands with 8 polymorphic bands and 20 common bands, where using SCoT marker produced a 28 total bands with 7 polymorphic bands Table 4 and PCR profile for RAPD, ISSR and SCoT was illustrated in Plate 4. Polymorphism percentage was highest 47.82% using RAPD marker and the lowest polymorphism using SCoT marker 25%

For *Limbarda crithmoides*, RAPD-PCR banding profile range illustrated in Plate 4. There are 23 total bands with 8 polymorphic bands and 15 common bands. ISSR primers produced a total 24 bands with 3 polymorphic bands and 21 common bands. SCoT marker gave 27 total bands with 7 polymorphic bands and 20 common bands. The highest polymorphism was generated using RAPD marker 34.78% and the lowest polymorphism was produced by using ISSR marker 12.5% Table 4.

- **Group B "seed size polymorphism":** For *Cakile maritima*, RAPD molecular marker produced profile banding shown in Plate 5. Total 29 bands with 9 polymorphic bands and 20 common bands were recorded. ISSR marker generated 28 total bands with 11 polymorphic bands and 17 common bands. The polymorphism varied from high percentage in case of SCoT marker 43.33% to low percentage using RAPD marker 31.03%

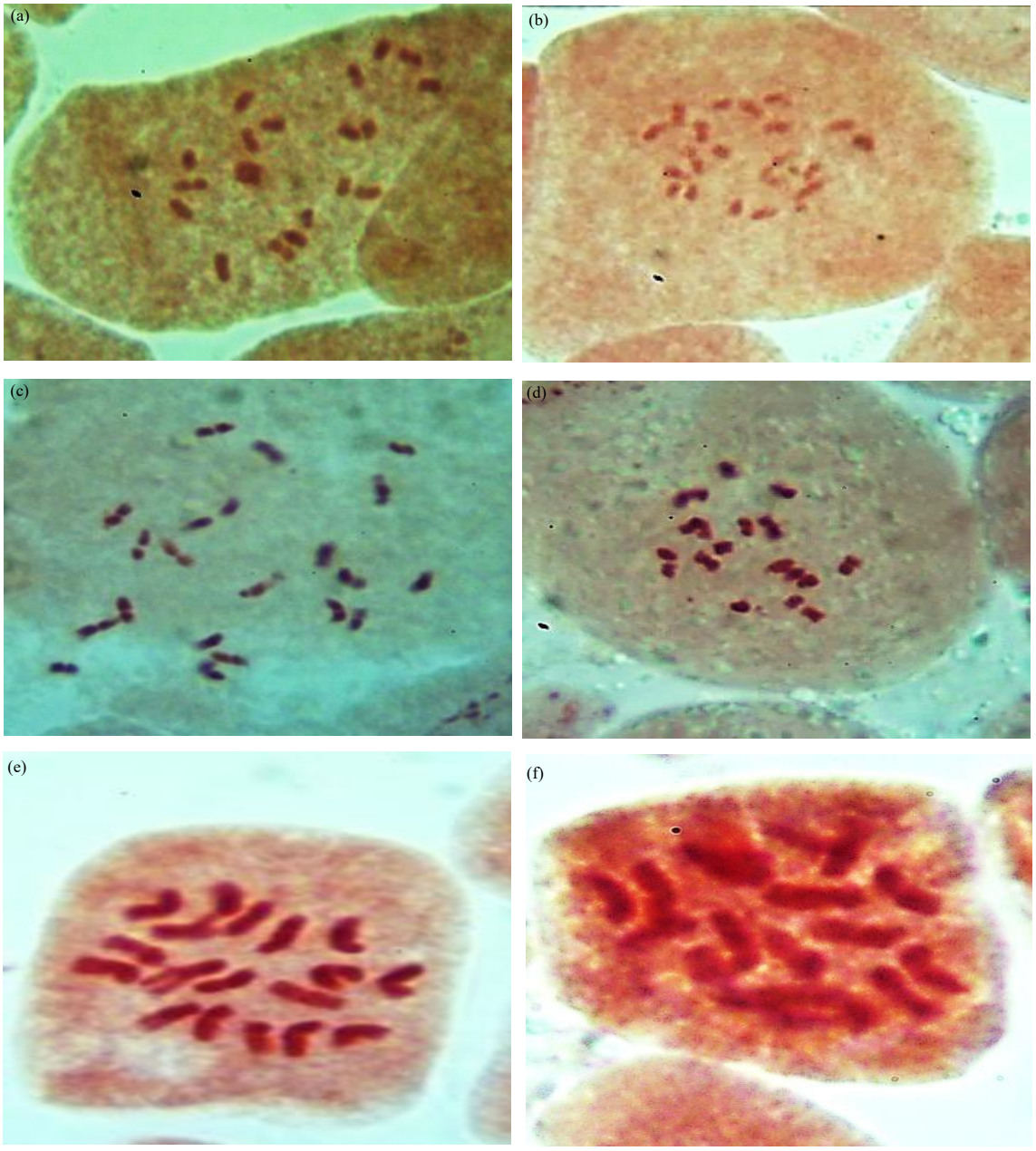

Plate 1(a-f): Chromosome number of group (A) "seed shape polymorphism, (a, b) Emex spinosa (aerial seed), (c, d) *Emex spinosa* (subterranean seed), (e) *Limbarda crithmoides* (monomorphic pappus) and (f) *Limbarda crithmoides* (dimorphic pappus)
X = 1000

For *Raphanus raphanistrum*, RAPD produced 32 total bands with 14 polymorphic bands and 18 common bands. In ISSR marker, 25 total bands were generated with 1 polymorphic band. In SCoT marker, 30 total bands were appeared with 7 polymorphic bands and 23 common bands. Polymorphism percentage ordered from 43.75% RAPD, 23.33% SCoT to 4% ISSR Table 4.

- **Group C "seed color polymorphism":** For *Chenopodium murale*, RAPD marker gave 22 total bands with 7 polymorphic bands and 15 common bands, where ISSR marker produced 22 total bands, there are 5 polymorphic bands and 17 common bands. SCoT marker generated 28 total bands with 2 polymorphic bands. The polymorphism percentage ranged from

Table 4: Total bands, polymorphic bands and percentage of polymorphism using RAPD, ISSR and SCoT markers

		Molecular markers											
		RAPD				ISSR				ScoT			
Groups	Taxa	Total bands	Polymorphic bands	Unique bands	Polymorphism (%)	Total bands	Polymorphic bands	Unique bands	Polymorphism (%)	Total bands	Polymorphic bands	Unique bands	Polymorphism (%)
A													
Seed shape	*Emex spinosa*	23	11	11	47.83	28	8	7	28.57	28	7	7	25.00
polymorphism	*Limbarda crithmoides*	23	8	8	34.78	24	3	3	12.50	27	7	7	29.93
B													
Seed size	*Cakile maritima*	29	9	9	31.03	28	11	11	39.19	30	13	13	43.33
Polymorphism	*Raphanus raphanistrum*	32	14	14	43.75	25	1	1	4.00	30	7	7	23.33
C													
Seed color	*Chenopodium murale*	22	7	7	31.82	22	5	5	22.73	28	2	2	7.14
polymorphism	*Urospermum picroides*	30	5	4	16.67	26	5	3	19.23	37	7	7	18.91
Total		159	54	53	33.96	153	33	31	21.57	180	43	43	23.89

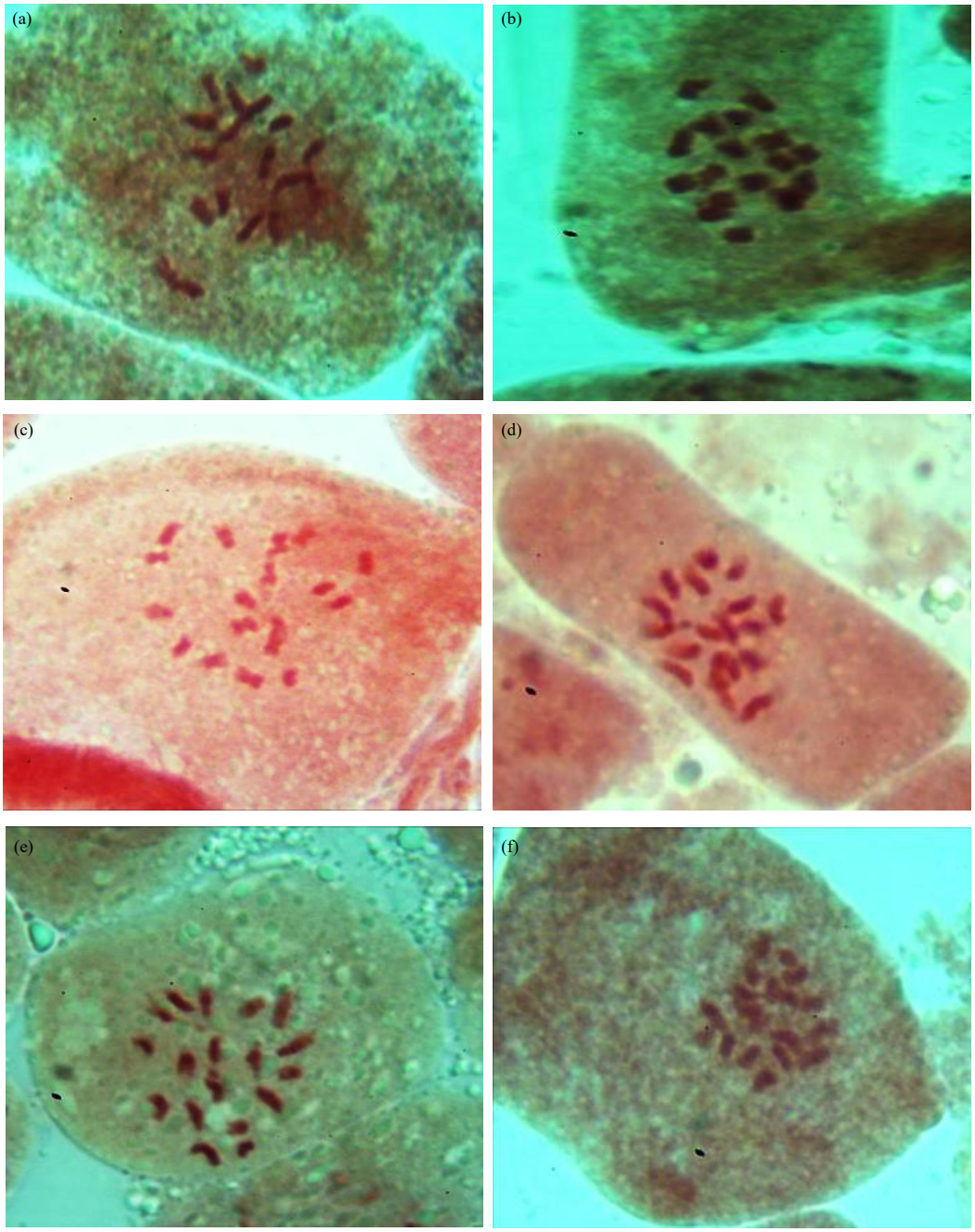

Plate 2(a-f): Chromosome number of group (B) "seed size polymorphism, (a, b) *Cakile maritima* (large seed), (c, d) *Cakile maritima* (small seed), (e) *Raphanus raphanistrum* (Large seed) and (f) *Raphanus raphanistrum* (small seed)
X = 1000

highest value 31.82% in using RAPD primers to lowest value 7.14% using SCoT primers. Banding profiles were observed in Plate 6

For *Urospermum picroides*, RAPD marker gave 30 total bands with 5 polymorphic bands and 25 common bands. From polymorphic bands there are 4 unique bands. In ISSR marker, 5 primers generated 26 total bands with 5 polymorphic bands and 3 unique bands. In SCoT marker, 37 total bands were appeared with 7 polymorphic bands and 30 common bands. In general RAPD molecular marker has the ability to differentiate among studied taxa produced polymorphism percentage 33.96% Table 4.

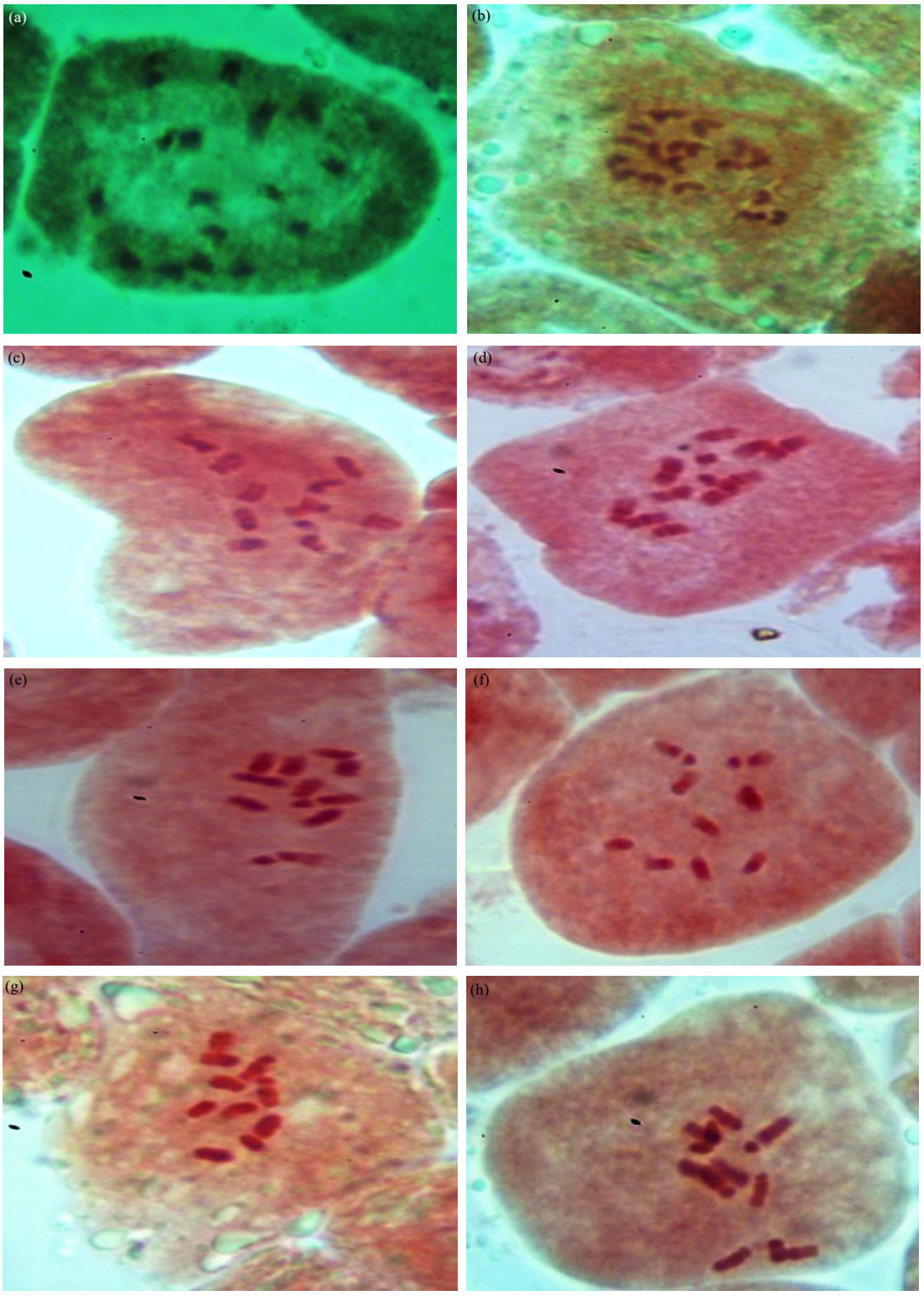

Plate 3(a-f): Chromosome number of group (C) "seed color polymorphism, (a) *Chenopodium murale* (black seed), (b) *Chenopodium murale* (brown seed), (c, d) *Urospermum picroides* (black seed), (e, f) *Urospermum picroides* (brown seed) and (g, h) *Urospermum picroides* (white seed)
X = 1000

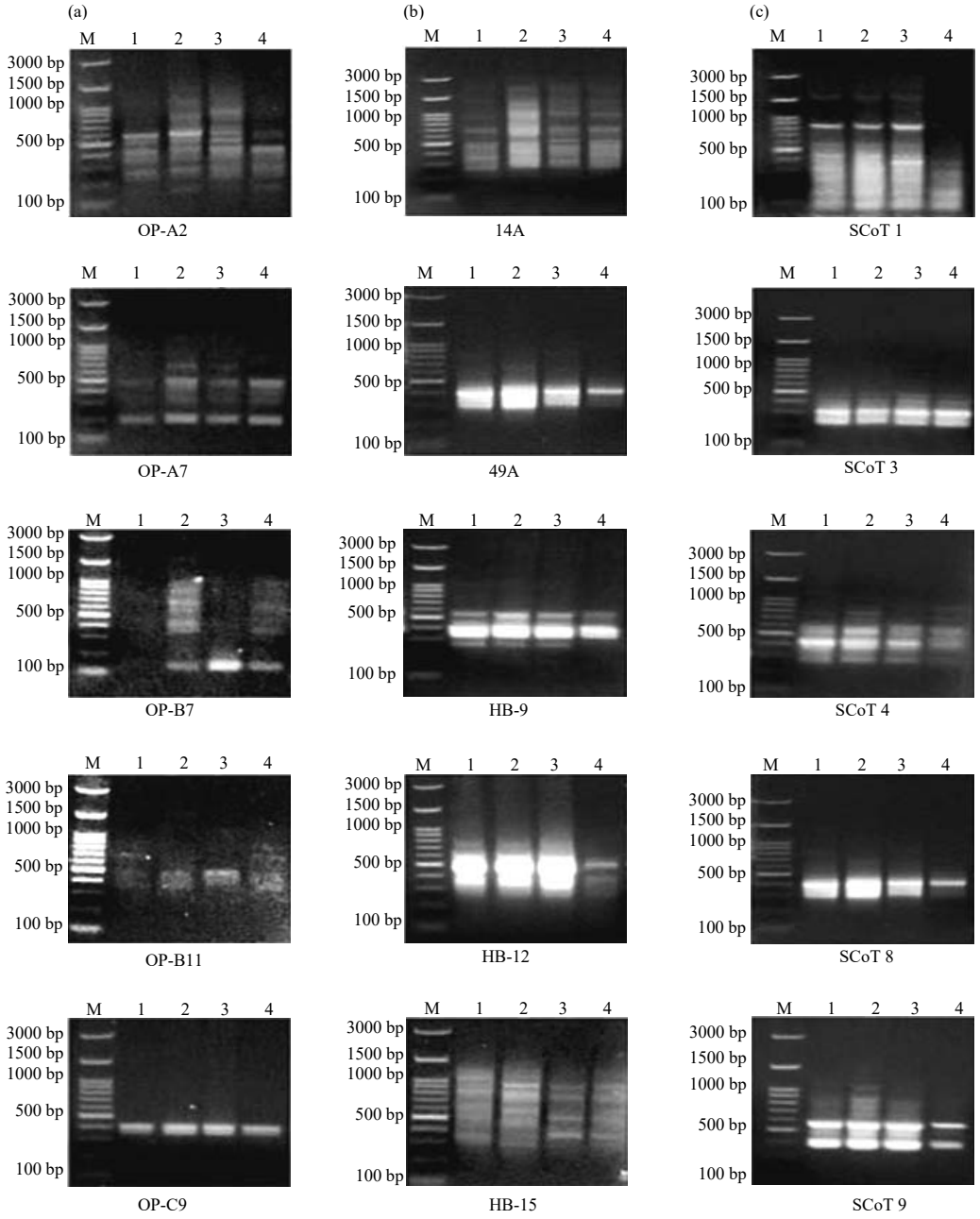

Plate 4(a-c): Banding profile of (a) RAPD, (b) ISSR and (c) SCoT markers for group (A)"seed shape polymorphism"
M: Marker, 1: *Emex spinosa* (aerial seed), 2: *Emex spinosa* (subterranean seed), 3: *Limbarda crithmoides* (monomorphic pappus), 4: *Limbarda crithmoides* (dimorphic pappus)

Scanning Electron Microscope (SEM): SEM micrographs were used to detect if there are any differences among distinct morphs of studied taxa which characterized by heterospermy as the following:

- **Group A: Seed shape polymorphism:** For *Emexspinosa*, subterranean seed revealed a globose ovate seed, the seed ended by 3 outer segments and terminated by pointed spines. Seed coat surface is irregular reticulated with epicuticular wax (Plate 7a, b). Aerial seed revealed an oval shaped with three-sided seed coat. Each side ended by a rigid curved spine. This seed has a head divided into 2 columns with tapered base and spiny. Seed coat surface showed a thick rough epicuticular (Plate 7c, d)

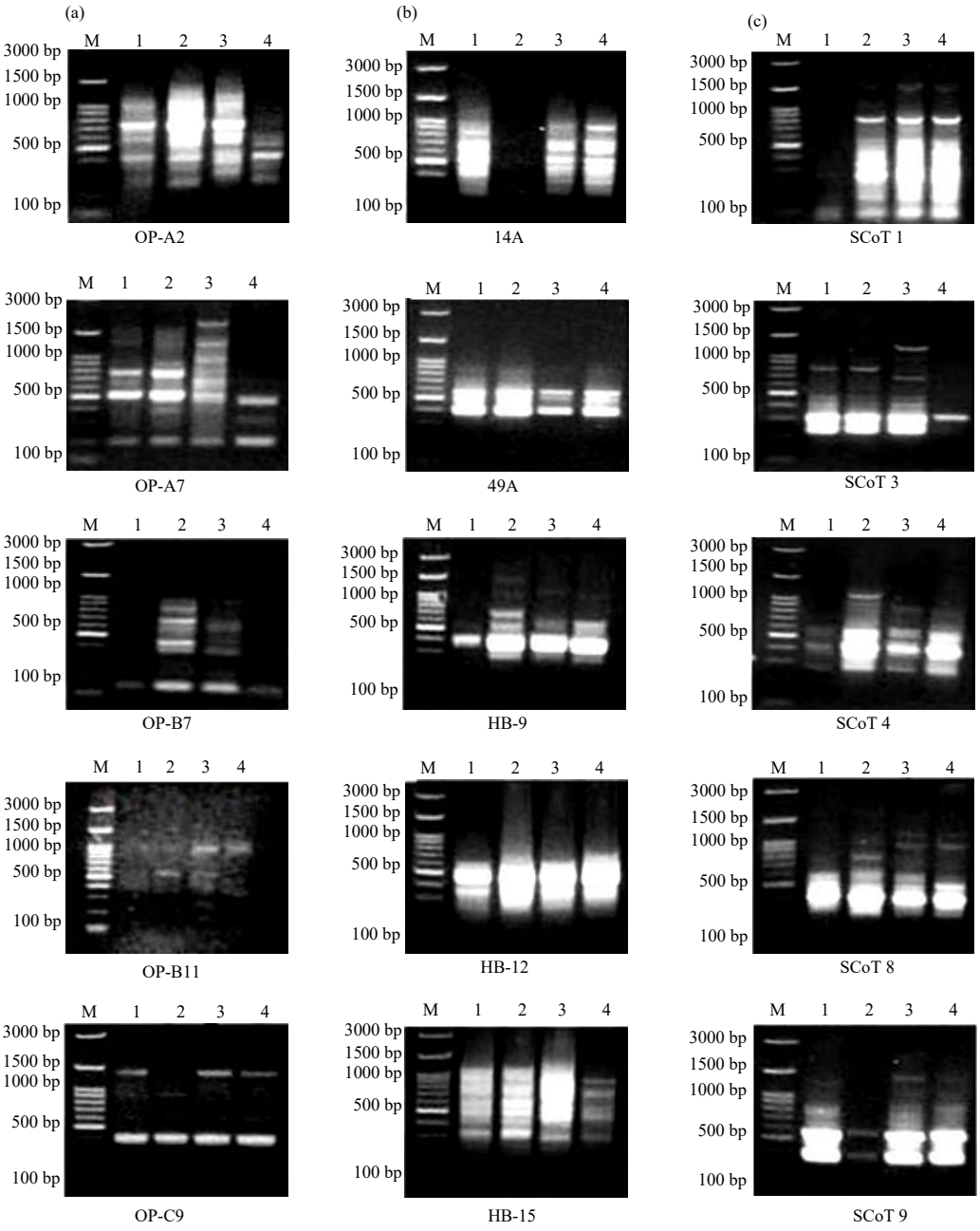

Plate 5(a-c): Banding profile of (a) RAPD, (b) ISSR and (c) SCoT markers for group (B)"seed size polymorphism"
M: Marker, 1: *Cakile maritima* (large seed), 2: *Cakile maritima* (small seed), 3: *Raphanus raphanistrum* (large seed), 4: *Raphanus raphanistrum* (small seed)

For *Limbarda crithmoides*, *Limbarda* with dimorphic pappus are appeared as angular shape with clearly hairs. Seed coat surface striated with thick waxy epicuticular cells forming ridges with warty wax and acute ended hairs (Plate 7e, f). *Limbarda* with monomorphic pappus appeared as oblong in shape with clearly hairs. Seed coat surface showed several rows of hairs with acute tip and warty wax (Plate 7g, h).

- **Group B seed size polymorphism:** For *Cakile maritima*, large seeds appeared as an obliquely ovate in shape with glabrous texture. Seed coat surface appeared as microreticulate structure with isodiametric and elongate with gonal epidermal cell shape (Plate 8a, b). Small seeds are oblong or ellipsoidal in shape with glabrous texture. Seeds have a central furrow and raised hilum. Seed coat

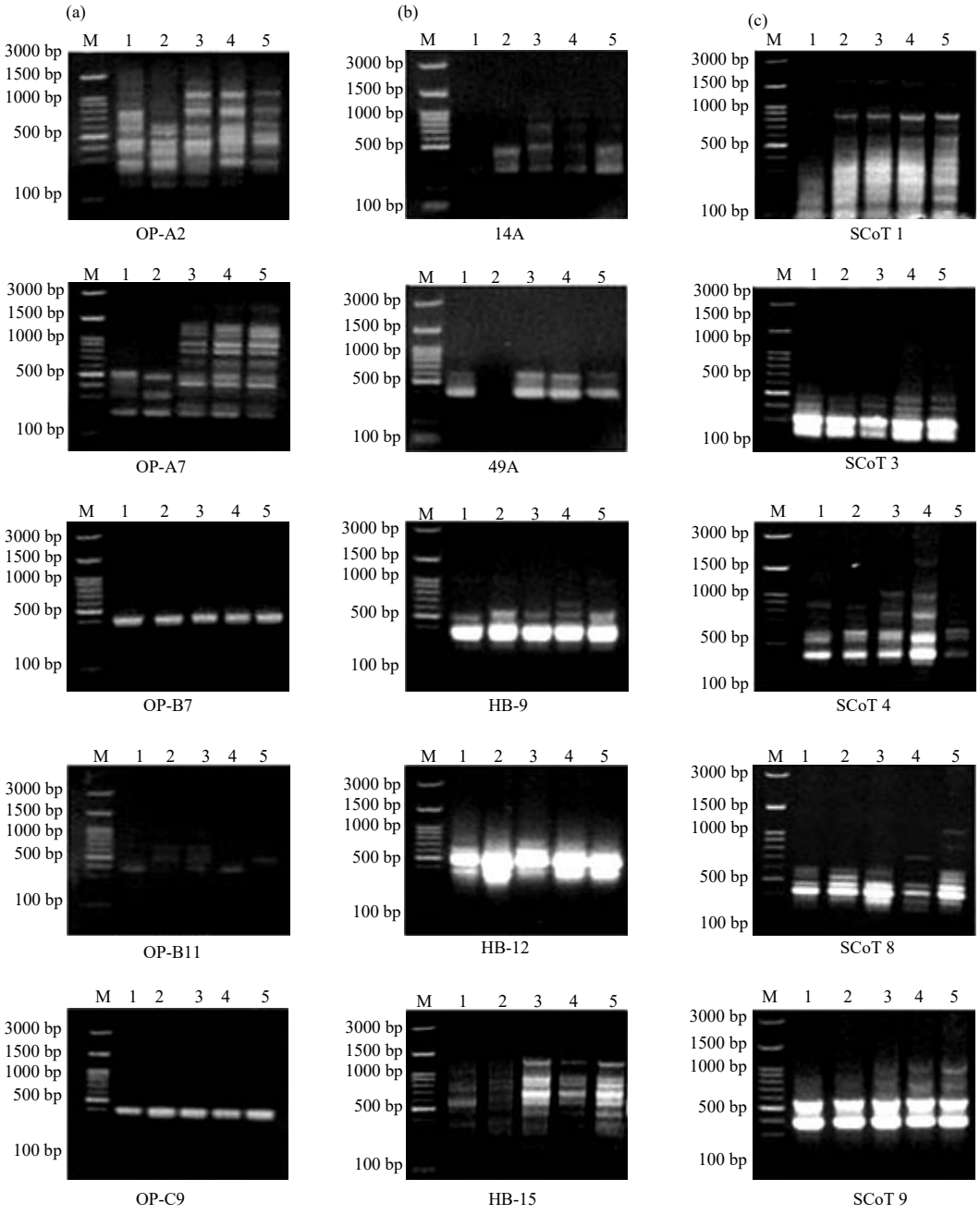

Plate 6(a-c): Banding profile of (a) RAPD, (b) ISSR and (c) SCoT markers for group (C)"seed color polymorphism"

M: Marker, 1: *Chenopodium murale* (black seed), 2: *Chenopodium murale* (brown seed), 3 *Urospermum picroides* (black seed), 4: *Urospermum picroides* (brown seed), 5: *Urospermum picroides* (white seed)

surface appeared as microreticulate structure with isodiametric and elongate with gonal epidermal cell shape (Plate 8c, d)

For *Raphanus raphanistrum*, large seeds of *Raphanus* appear as spherical in shape with a sided ridge. The magnified seed surface showed polygonal epidermal cells, the highly raised, rough and thin anticlinal walls with warty wax and flattened microreticulate pitted periclinal wall (Plate 8e, f). Small seeds of *Raphanus* are spherical in shape. Seed coat surface is reticulated with small and shallow inter spaces. The magnified seeds showed a reticulate surface with raised thick rough anticlinal walls and papillated, microreticulated pittetted and periclinal walls (Plate 8g, h).

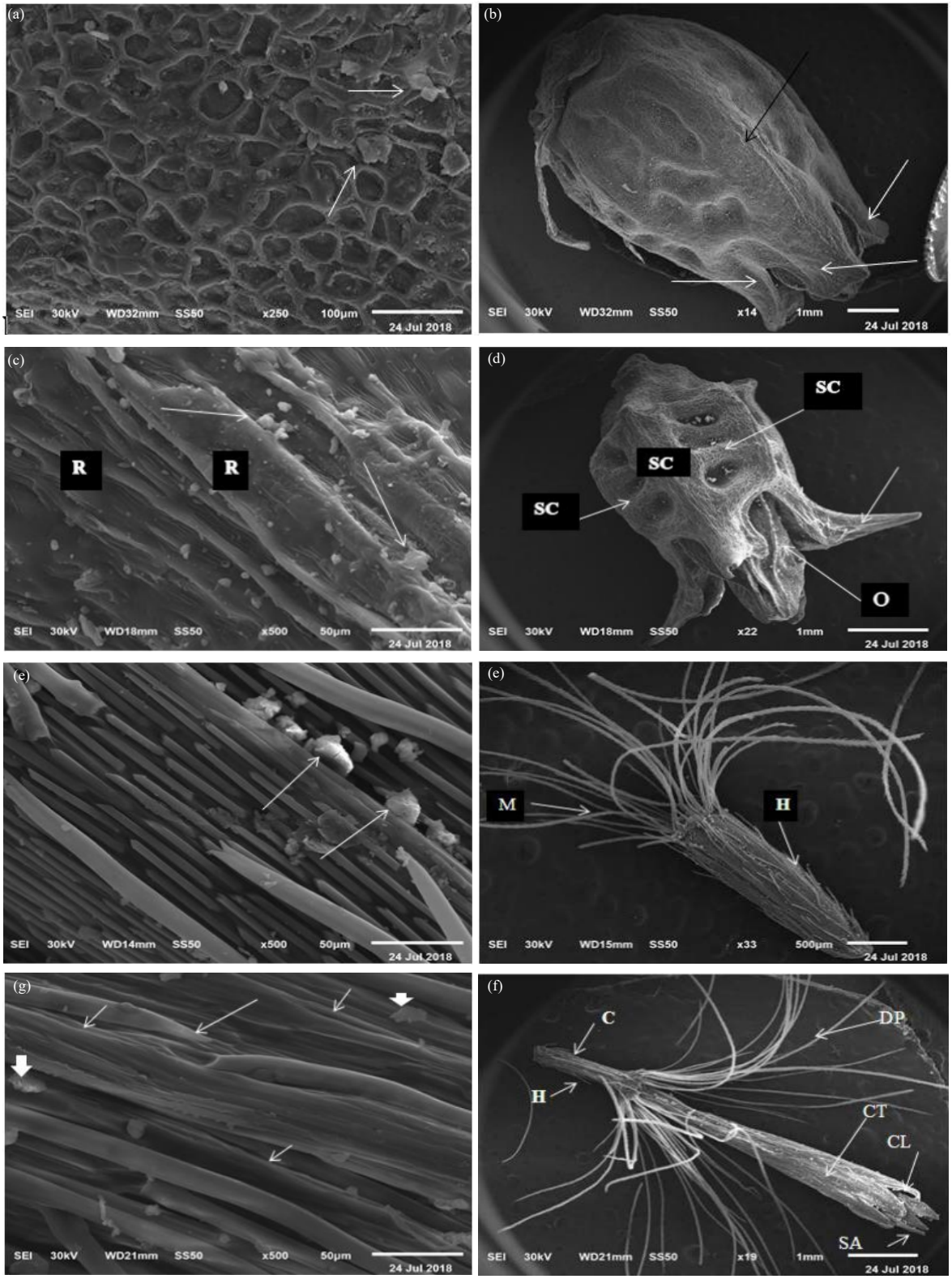

Plate 7(a-h): SEM micrograph of seed morphology and coat for Group A, (a, b): *Emey spinosa* aerial seed, (c, d): *Exem spinosa* (subterranean seed), (e, f) *Limbarda crithmoides* (monomorphic pappus) and (g, h): *Limbarda crithmoides* (dimorphic pappus)

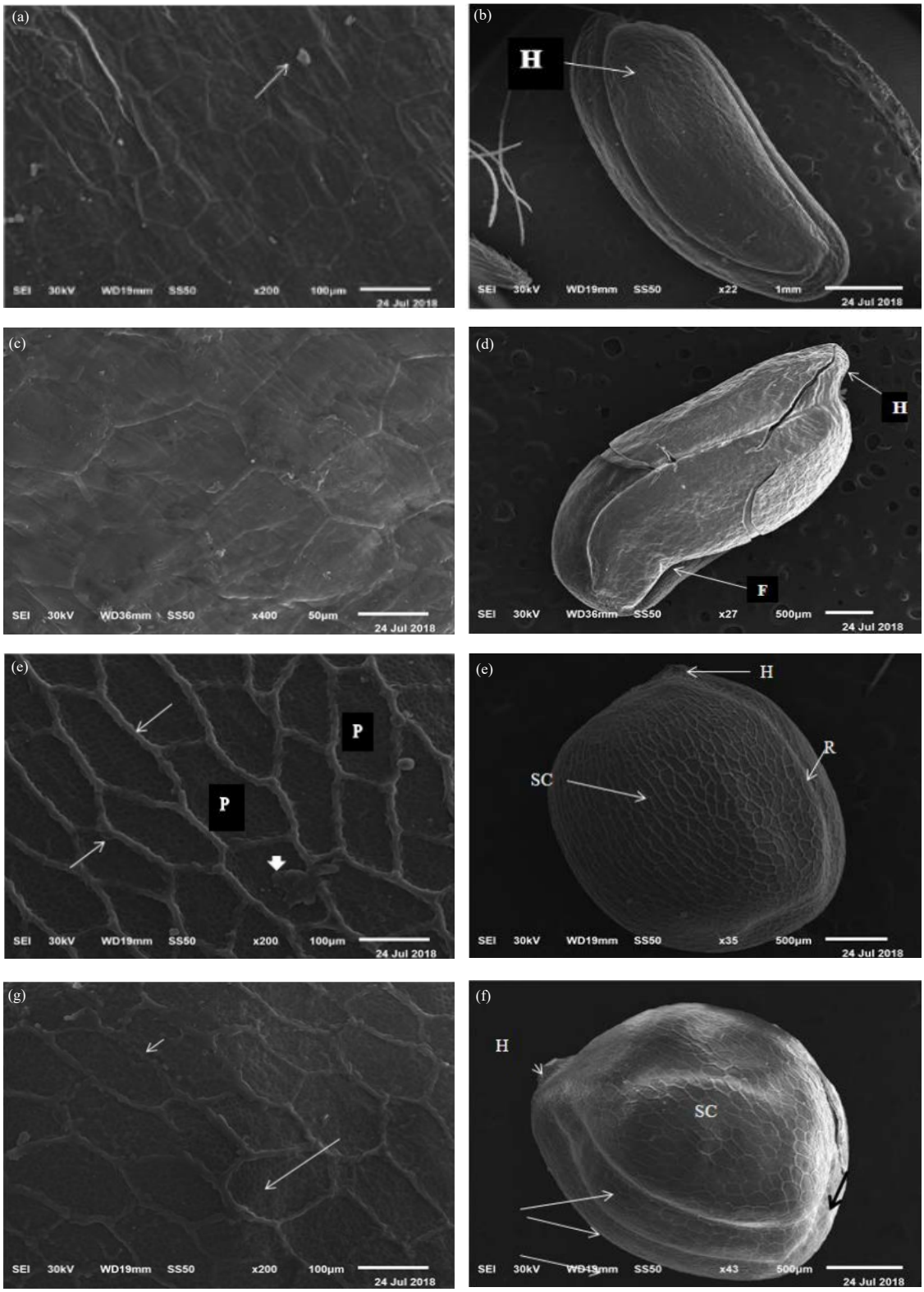

Plate 8(a-h): SEM micrograph of seed morphology and coat for Group B, (a, b) *Cakile maritima* (large seed), (c, d) *Cakile maritima* (small seed), (e, f) *Raphanus raphanistrum* (large seed) and (g, h) *Raphanus raphanistrum* (small seed)

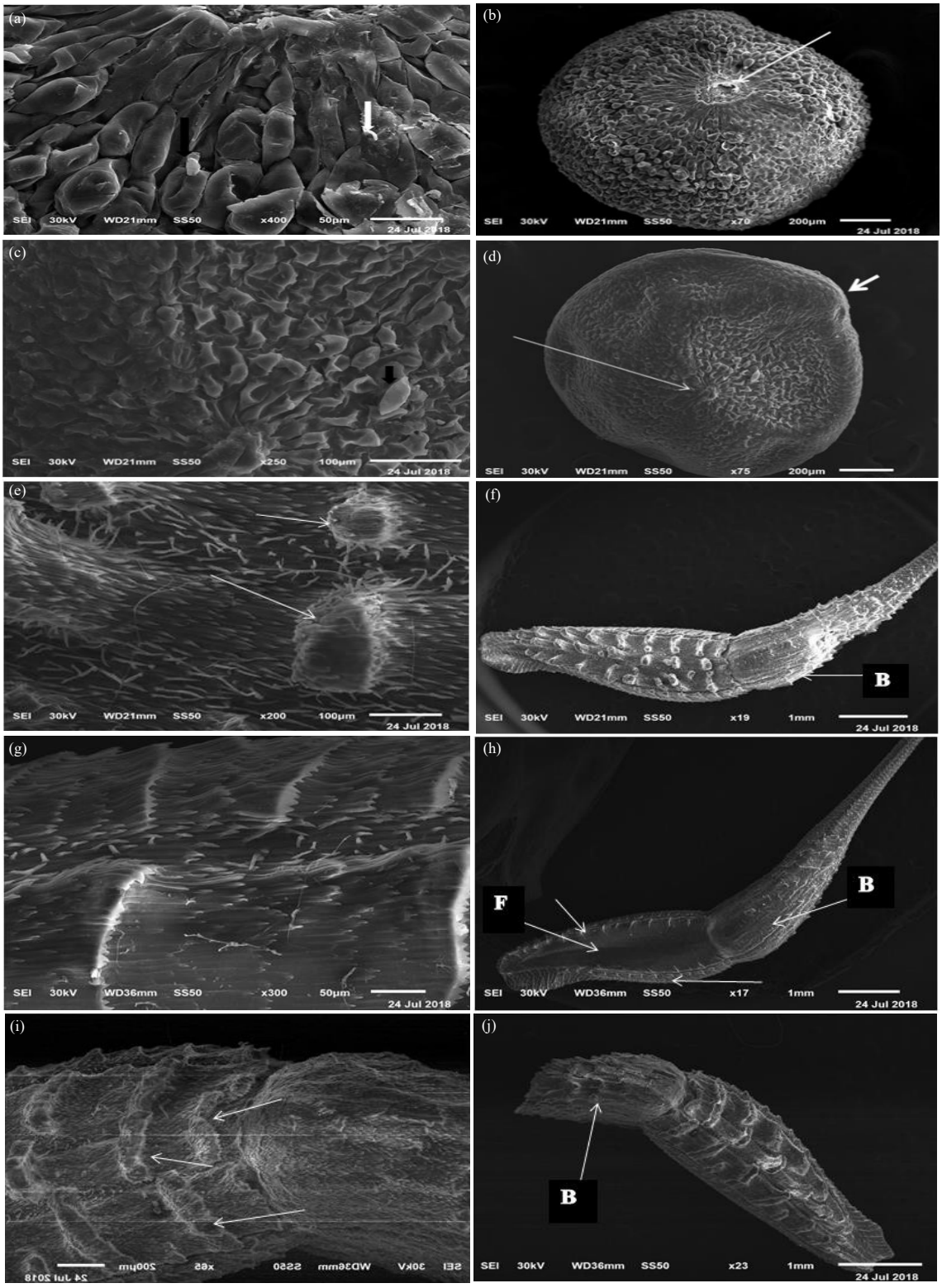

Plate 9(a-j): SEM micrograph of seed morphology and coat for Group (B), (a, b) *Chenopodium murale* (blackseed), (c, d) *Chenopodium murale* (brown seed), (e, f) *Urospermum picroides* (black seed), (g, h) *Urospermum picroides* (brown seed) and (I, j) *Urospermum picroides* (white seed)

- **Group C seed colour polymorphism:** For *Chenopodium murale*, black seed appears as spherical in shape with styler scar and tuberculated surface of testa. The magnified part of seed surface showed irregularly and shallowly tuberculated ornamentation on seed surface with glandular trichome and simple curved hairs (Plate 9a, b) Brown seed of *Chenopodium murale* appear as sub-spherical shaped compressed into multi sided prominence with shallow hilar notch which is seen along the periphery and styler scar. The magnified part of seed surface showed irregular undulate ornamentation of wax on pericarp surface with glandular trichomes (Plate 9c, d)

For *Urospermum picroides*, there are 3 different colour of *Urospermum picroides.* Black seed of *Urospermum picroides* appear as oblong shaped with beak. The beak is tapered, the thick part is toward the body of seed and the thin part is outside. The beak is longer than the body of the seed. The seed surface is rough and papillose (Plate 9e, f). Brown seed of *Urospermum picroides* appear as oblong shaped. One furrow is present and surround by two raised papillate ridges. Long beak with tapering end which is far from the seed body and the thick part is attached with the seed body (Plate 9g, h). White seed of *Urospermum picroides* is oblong in shape with beak. The seed surface is rough and papillose. The papillae covered most of the seed surface and the beak is thick and short (Plate 9i, j).

DISCUSSION

Genetic variation is an important tool in gene bank management, helping in the establishment of core collections, facilitating efficient sampling and utilization of germ plasm and selecting of desirable genotypes to be used in breeding programs[33].

Chromosomal diversity is a key factor contributing to genetic, phenotypic and ecological evolution in Angiosperms. Chromosomal diversity can be expressed in a wide range of numerical, morphological and molecular features[19]. In systematics, chromosome number is an important character for plant evolutionary studies and may provide some information about polyploidy and other highly significant genome changes[34,35]. Chromosome numbers for all seed forms of three groups, seed shape, seed size and seed color polymorphism were agree with previous account except in case of *Emex spinosa* 2n = 20 disagree with previous count 2n = 18 by Abd El-Twab *et al.*[29].

In this study, there was any variation in chromosome number in all seed forms for three groups of seed polymorphism, the change appeared in the chromosome structure, shape and thickness of chromosome may be due to difference in chromosome arm condensation[36] or due to long period of genetic isolation[37,38].

The advantage of molecular markers over phenotypic data is the possibility to compare genotypes, even if they are sampled in different environment, type of tissue or stage of development. Another advantage is the theoretical possibility to detect DNA polymorphisms through the entire genome[39].

In this study, DNA molecular markers are independent of environmental conditions or developmental stage and showed high level of polymorphism. Molecular analysis in this study confirmed the outcomes of morphological and taxonomic analyses done on the collected samples. For seed shape polymorphism, RAPD polymorphism percentage in *Emex spinosa* 47.83% is higher than polymorphism percentage in pear cultivars of Polygonaceae (46.15%) using the same primers of RAPD by El-Hawary *et al.*[40]. On the other hand, SCoT primers generated 25 total bands and 7 polymorphic bands for *Emex spinosa* compared to cultivated pear having 23 total bands and 10 polymorphic bands using the same 5 primers of SCoT marker by El-Hawary *et al.*[40] In seed size polymorphism, unique bands were 9 for *Cakile maritima* compared to unique bands were 2 in *Cakile maritima* by Mohamed[41]. Five RAPD primers generated 9 total bands for *Cakile maritima* and 14 total bands for *Raphanus raphanistrum* and compared to 614 total bands using 74 RAPD primers for *Brassica oleracea* by Lu *et al.*[42].

For RAPD analysis in *Chenopodium murale*, there are 22 total bands using RAPD marker and 28 total bands for SCoT primers compared to *Chenopodium quinoa* having 64 total bands for RAPD and 79 total band using SCoT primers by Lema-Rumińska *et al.*[43].

The ISSR analysis for *Urospermum picroides* produced 30 total bands with 16.67% lower than polymorphism 52.77% generated from RAPD marker in *Acmella paniculata* by Gupta *et al.*[44]. Finally RAPD molecular marker has the ability to differentiate among studied taxa showing 33.96% than ISSR and SCoT markers.

For conclusion, it was illustrated that RAPD marker were found to be more efficient than ISSR and SCoT markers with regards to polymorphism detection.

Seed characters are stable and less affected by environmental conditions while the seeds develop and ripen within the fruit[45] and often show genetic differences. Some investigations suggested that seed surface pattern is

conservative trait and can be considered as genetic markers as it is a vital value in taxonomic delimitations and in hybridization[46]. Very few researches have been undertaken on the seed coat surface of the species characterized by heterospermy. SEM analysis of the seed coat surface and seed test a of *Emex spinosa* revealed that coat surface of aerial seed characterized by sulcate coat patterns with a thick rough epicuticular wax and wax granules. Also, Gabr[47] reported the sulcate coat patterns of seeds of *Centaurea aegyptiaca* and *Launaea nudicaulis*. The coat surface of subterranean seed is irregularly reticulate coat patterns with a thick rough epicuticular wax and wax granules which is similar to results of Kasem *et al*.[48], who revealed the irregular reticulate coat patterns in *Brassica oleracea* seed. The SEM micrographs of *Limbarda crithmoides* seeds showed that the coat surface of seed with monomorphic pappus is aculeate coat patterns and its surface covers with several rows of hairs. This agreed with the results of Gabr[49], who investigated aculeate coat patterns of the *Asclepias curassavica* seed surface.

The SEM analysis of the seed coat surface and seed test a of *Chenopodium murale* revealed that coat surface of black and brown seeds are different. This result disagreed with Devi and Chrungoo[50], who recorded 2 different seed coat surfaces of *Chenopodium album*, smooth seed coat (brown seed cultivar) and reticulated seed coat in (black seed cultivar). The SEM micrographs of *Urospermum picroides* seeds (black, brown and white) showed its oblong shape. This result agreed with Sadeq and Aliwy[51], who recorded that the surface of *Urospermum picroides* seed coat is papillae covered with long narrowed scales with pointed end, also Gabr[47], indicated the reticulated seed coat sculpture of *Urospermum picroides*.

CONCLUSION

The heteromorphism phenomena in the studied three group of seed polymorphism (seed shape, seed size and seed color) may be due to genetic variations during the formation period of seeds. Therefore, seed polymorphism considered a very important tool in plant conservation and should be take into consideration during collection stages of seeds for conservation of plant genetic resources in gene banks.

SIGNIFICANCE STATEMENT

This study discovered the importance of seed heterospermy phenomena (seed shape, seed size and seed color polymorphism) in genetic diversity. The seeds of the same plant with heterospermy have the same number of chromosome number but differed genetically in both chromosome structure and DNA fingerprinting. This study will help the researchers to take into consideration this phenomenon in plant conservation and genetic diversity.

REFERENCES

1. Clauss, M.J. and D.L. Venable, 2000. Seed germination in desert annuals: An empirical test of adaptive bet hedging. Am. Nat., 155: 168-186.
2. Imbert, E., 2002. Ecological consequences and ontogeny of seed heteromorphism. Perspect. Plant Ecol. Evol. Syst., 5: 13-36.
3. Wang, L., M. Dong and Z. Huang, 2010. Review of research on seed heteromorphism and its ecological significance. J. Plant Ecol., 34: 578-590.
4. Mandak, B. and P. Pys, 2005. How does seed heteromorphism influence the life history stages of *Atriplex sagittata* (Chenopodiaceae)? Flora-Morphol. Distrib. Funct. Ecol. Plants, 200: 516-526.
5. Bhatt, A. and A. Santo, 2016. Germination and recovery of heteromorphic seeds of *Atriplex canescens* (Amaranthaceae) under increasing salinity. Plant Ecol., 217: 1069-1079.
6. Emura, N., T. Denda, M. Sakai and K. Ueda, 2014. Dimorphism of the seed-dispersing organ in a pantropical coastal plant, *Scaevola taccada*: Heterogeneous population structures across islands. Ecol. Res., 29: 733-740.
7. Miguel, M.F., C.J. Lortie, R.M. Callaway and J.L. Hierro, 2017. Competition does not come at the expense of colonization in seed morphs with increased size and dispersal. Am. J. Bot., 104: 1323-1333.
8. Gul, B., R. Ansari, T.J. Flowers and M.A. Khan, 2013. Germination strategies of halophyte seeds under salinity. Environ. Exp. Bot., 92: 4-18.
9. Mandák, B. and P. Pyšek, 2001. The effects of light quality, nitrate concentration and presence of bracteoles on germination of different fruit types in the heterocarpous *Atriplex sagittata*. J. Ecol., 89: 149-158.
10. Einum, S. and I.A. Fleming, 2002. Does within population variation in fish egg size reflect maternal influences on optimal values? Am. Nat., 160: 756-765.
11. Simons, A.M. and M.O. Johnston, 2000. Variation in seed traits of *Lobelia inflata* (Campanulaceae): Sources and fitness consequences. Am. J. Bot., 87: 124-132.
12. Barbour, M.G., 1972. Seedling establishment of *Cakile maritima* at Bodega Head, California. Bull. Torrey Bot. Club, 99: 11-16.
13. Barbour, M.G., 1970. Germination and early growth of the strand plant *Cakile maritima*. Bull. Torrey Bot. Club, 97: 13-22.
14. Cordazzo, C.V., 2006. Seed characteristics and dispersal of dimorphic fruit segments of *Cakile maritima* Scopoli (Brassicaceae) population of southern Brazilian coastal dunes. Braz. J. Bot., 29: 259-265.

15. Stanton, M.L., 1984. Developmental and genetic sources of seed weight variation in *Raphanus raphanistrum* L. (Brassicaceae). Am. J. Bot., 71: 1090-1098.
16. Yao, S., H. Lan and F. Zhang, 2010. Variation of seed heteromorphism in Chenopodium album and the effect of salinity stress on the descendants. Ann. Bot., 105: 1015-1025.
17. Williams, J.T. and J. L. Harper, 1965. Seed polymorphism and germination. I. The influence of nitrates and low temperatures on the germination of *Chenopodium album*. Weed Res., 5: 141-150.
18. Lysak, M.A., A. Berr, A. Pecinka, R. Schmidt, K. McBreen and I. Schubert, 2006. Mechanisms of chromosome number reduction in Arabidopsis thaliana and related Brassicaceae species. Proc. Natl. Acad. Sci. USA., 103: 5224-5229.
19. Weiss-Schneeweiss, H. and G.M. Schneeweiss, 2013. Karyotype Diversity and Evolutionary Trends in Angiosperms. In: Plant Genome Diversity: Physical Structure, Behavior and Evolution of Plant Genomes, Greilhuber, J., J. Dolezel and J.F. Wendel (Eds.)., Volume 2, Springer, Vienna, ISBN: 978-3-7091-1159-8, pp: 209-230.
20. Govindaraj, M., M. Vetriventhan and M. Srinivasan, 2015. Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. Genet. Res. Int., Vol. 2015. 10.1155/2015/431487.
21. Gorji, A.M., P. Poczai, Z. Polgar and J. Taller, 2011. Efficiency of arbitrarily amplified dominant markers (SCoT, ISSR and RAPD) for diagnostic fingerprinting in tetraploid potato. Am. J. Potato Res., 88: 226-237.
22. Madhou, P., A. Wells, E.C.K. Pang and T.W. Stevenson, 2005. Genetic variation in populations of Western Australian wild radish. Aust. J. Agric. Res., 56: 1079-1087.
23. Huh, M.K. and O. Ohnishi, 2002. Genetic diversity and genetic relationships of east asian natural populations of wild radish revealed by AFLP. Breed. Sci., 52: 79-88.
24. Brisson, J.D. and R.L. Peterson, 1976. A critical review of the use of scanning electron microscopy in the study of the seed coat. Proceedings of the Workshop on Plant Science Applications of the Scanning Electron Microscope, April 1976, IIT Research Institute, Chicago, pp: 477-495.
25. Mostafavi, G., M. Assadi, T. Nejadsattari, F. Sharifnia and I. Mehregan, 2013. Seed micromorphological survey of the Minuartia species (Caryophyllaceae) in Iran. Turk. J. Bot., 37: 446-454.
26. Chattopadhyay, D. and A.K. Sharma, 1988. A new technique for orcein banding with acid treatment. Stain Technol., 63: 283-287.
27. Dellaporta, S.L., J. Wood and J.B. Hicks, 1983. A plant DNA minipreparation: Version II. Plant Mol. Biol. Rep., 1: 19-21.
28. Malallah, G.A., M. Al-Dosari and A. Murin, 2001. Determination of chromosome numbers in Kuwaiti flora II. Thaiszia J. Bot., 10: 137-150.
29. Abd El-Twab, M.H., A.M. Abdel-Hamid and H.A.A. Mohamed, 2012. Karymorphological and molecular studies on seven species in Polygonoideae (Polygonaceae) in Egypt. Chromosome Bot., 7: 17-22.
30. Soliman, M.I., 2002. Karyological studies on some wild species of family cruciferae in Egypt. Pak. J. Biol. Sci., 5: 943-947.
31. Benabdelmouna, A., G. Guéritaine, M. Abirached-Darmency and H. Darmency, 2003. Genome discrimination in progeny of interspecific hybrids between *Brassica napus* and *Raphanus raphanistrum*. Genome, 46: 469-472.
32. Abd-El-Wahid, A.A., M.I. Soliman, R.M. Rizk and R.M. Rizk, 2009. Cytogenetical studies on achene colour polymorphism of *Picris asplenoides* L. and *Urospermum picroides* L. (Asteraceae) in Egypt. Pak. J. Biol. Sci., 12: 565-573.
33. Radwan, S.A., A.S. El-Kholy and R.H. Sammour, 2013. Genetic variation among accessions of *Lathyrus inconspicuous* (L.) as revealed by SDS polyacrylamide gel electrophoresis. Acta Agric. Solvenica, 101: 21-30.
34. Guerra, M., 2008. Chromosome numbers in plant cytotaxonomy: Concepts and implications. Cytogenet. Genome Res., 120: 339-350.
35. Louzada, R.B., C. Palma-Silva, A.M. Corrêa, E. Kaltchuk-Santos and M.D.G.L. Wanderley, 2010. Chromosome number of *Orthophytum* species (Bromeliaceae). Kew Bull., 65: 53-85.
36. Kakeda, K. and K. Fukui, 1994. Dynamic changes in the morphology of barley chromosomes during the mitotic metaphase stage. Jpn. J. Genet., 69: 454-554.
37. Briggs, D. and S.M. Walters, 1984. Plant Variation and Evolution. 2nd Edn., Cambridge University Press, New York.
38. Soliman, M.I., 2003. Genetic diversity of achene heteromorphism in egyptian *Calendula micrantha* tineo et guss. Asian J. Plant Sci., 2: 782-789.
39. Sakiyama, N.S., H.C.C. Ramos, E.T. Caixeta and M.G. Pereira, 2014. Plant breeding with marker-assisted selection in Brazil. Crop Breed. Applied Biotechnol., 14: 54-60.
40. El-Hawary, S.S., M.E. El-Tantawy, F.N. Kirollos and E. Hammam, 2018. Inter Simple Sequence Repeat (ISSR) and Start Codon Targeted polymorphism (Scot) as discrimination techniques between certain apple and pear cultivars. Int. Res. J. Pharm., 9: 80-86.
41. Mohamed, A.S., 2009. Molecular systematic of some brassicaceae taxa in egypt based on electrophoretic isoenzymes pattern and rapd markers. Aust. J. Basic Applied Sci., 3: 1499-1511.
42. Lu, X.J., L.W. Liu, Y.Q. Gong, L.P. Zhao, X.Y. Song and X.W. Zhu, 2009. Cultivar identification and genetic diversity analysis of broccoli and its related species with RAPD and ISSR markers. Scient. Hortic., 122: 645-648.
43. Lema-Rumińska, J., N. Miler and K. Gęsiński, 2018. Identification of new polish lines of *Chenopodium quinoa* (willd.) by spectral analysis of pigments and a confirmation of genetic stability with scot and rapd markers. Acta Sci. Pol. Hortorum Cultus, 17: 75-86.

44. Gupta, D.D., P.K. Hui and H. Tag, 2017. Molecular characterization of *Acmella paniculata* (Asteraceae) from Arunachal Himalayan region through RAPD and ISSR markers. J. Basic Applied Plant Sci., Vol. 1.
45. Barthlott, W., 1981. Epidermal and seed surface characters of plants: Systematic applicability and some evolutionary aspects. Nordic J. Bot., 1: 345-355.
46. Kumar, D. and N.S. Rangaswamy, 1984. SEM studies on seed surface of wild and cultivated species of *Vigna savi*. Proc. Plant Sci., 93: 35-42.
47. Gabr, D.G., 2015. Comparative morphological studies on achene of some taxa of asteraceae. Arab Univ. J. Agric. Sci., 23: 601-614.
48. Kasem, W.T., A. Ghareeb and E. Marwa, 2011. Seed morphology and seed coat sculpturing of 32 taxa of family Brassicaceae. J. Am. Sci., 7: 166-178.
49. Gabr, D.G., 2014. Seed morphology and seed coat anatomy of some species of Apocynaceae and Asclepiadaceae. Ann. Agric. Sci., 59: 229-238.
50. Devi, R.J. and N.K. Chrungoo, 2017. Evolutionary divergence in Chenopodium and validation of SNPs in chloroplast rbcL and matk genes by allele-specific PCR for development of Chenopodium quinoa-specific markers. Crop J., 5: 32-42.
51. Sadeq, Z.G. and S.A. Aliwy, 2019. Micro-morphology study of pollen grains and cypsela of seven selected species, belong to asteraceae family in Al-Jadriya campus. Iraqi J. Agric. Sci., 50: 1138-1152.